Rec'd PCT/PTO 19 MAY 1999

FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. P8341-9011
	TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	DATE: May 19, 1999
		U.S. APPLN. NO. (IF KNOWN, SEE 37 CFR 1.5) 09/308223

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP97/06452	19 NOVEMBER 1997	19 NOVEMBER 1996

TITLE OF INVENTION: STABLE LYOPHILIZED PHARMACEUTICAL SUBSTANCES FROM MONOCLONAL OR POLYCLONAL ANTIBODIES

APPLICANT(S) FOR DO/EO/US: Georg KALLMEYER, Gerhard WINTER, Christian KLESSEN, Heinrich WOOG

1. XX This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
   (THE BASIC FILING FEE IS ATTACHED)

2. _ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. XX This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT articles 22 and 39(1).

4. XX A proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. XX A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. XX is transmitted herewith (required only if not transmitted by the International Bureau).
   b. XX has been transmitted by the International Bureau.
   c. _ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. XX A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. _ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. _ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. _ have been transmitted by the International Bureau.
   c. _ have not been made; however, the time limit for making such amendments has NOT expired.
   d. _ have not been made and will not be made.

8. _ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. _ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. _ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. XX An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. _ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. XX A FIRST preliminary amendment.
    _ A SECOND or SUBSEQUENT preliminary amendment.

14. _ A substitute specification.

15. _ A change of power of attorney and/or address letter.

16. XX Other items or information: PCT/ISA/220, PCT/ISA/210, PCT/IPEA/401, PCT/RO/105, PCT/RO/101, PCT/IB/306, PCT/IPEA/416, PCT/IPEA/409
    CHECK NO. 19633

U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO. PCT/EP97/06452	ATTORNEY DOCKET NO. P8341-9011 DATE: May 19, 1999

17. XX The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5):

Search Report has been prepared by the EPO or JPO.....$840.00
International preliminary examination fee paid to USPTO (37 CFR 1.482)...$670.00
No international preliminary examination fee paid to USPTO (37 CFR1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))....$760.00
Neither international preliminary examination fee (37 CFR 1.482) or international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$970.00
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....$ 96.00

	CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840	
Surcharge of $130.00 for furnishing the oath or declaration later than _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	24 - 20 =	04	X $ 18.00	$72	
Independent Claims	03 - 3 =	00	X $ 78.00	$00	
Multiple dependent claim(s) (if applicable)			+ $260.00	$00	
TOTAL OF ABOVE CALCULATIONS =				$912	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$00	
SUBTOTAL =				$912	
Processing fee of $130.00 for furnishing the English translation later the _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$00	
TOTAL NATIONAL FEE =				$912	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$00	
TOTAL FEES ENCLOSED =				$840	
				Amount to be refunded	$
				Charged	$

a. XX A check in the amount of $840 to cover the above fees is enclosed.
b. _ Please charge my Deposit Account No. 14-1060 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. XX The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1060.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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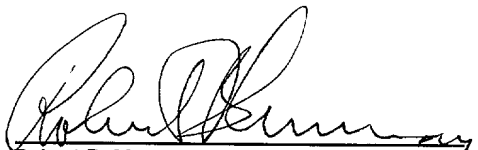

Robert B. Murray
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Georg KALLMEYER et al

Serial No.: Unknown

Filed: May 19, 1999

For: STABLE LYOPHILIZED PHARMACEUTICAL SUBSTANCES FROM MONOCLONAL OR POLYCLONAL ANTIBODIES

PRELIMINARY AMENDMENT

Assistant Commissioner
for Patents
Washington, D.C. 20231

May 19, 1999

Sir:

Prior to calculation of the filing fee and prior to the examination of this application, please amend the above-identified application as follows:

IN THE CLAIMS:

Please cancel claims 1-12 and insert the following new claims:

--13. A lyophilizate, comprising

(1) a monoclonal antibody or a polyclonal antibody;

(2) a sugar or an amino sugar;

(3) at least one amino acid; and

(4) a surfactant.

14. The lyophilizate of claim 13, wherein the lyophilizate is essentially free of polyethylene glycols and/or proteinaceous pharmaceutical auxiliary additives.

15. The lyophilizate of claim 13, wherein the lyophilizate contains a single amino acid or two different amino acids.

16. The lyophilizate of claim 13, further comprising a buffering agent or an isotonizing agent.

17. The lyophilizate of claim 13, wherein the lyophilizate is storage-stable for a time period of at least three months at a temperature of about 4-12°C.

18. The lyophilizate of claim 13, wherein the lyophilizate is storage-stable for a time period of at least three months at a temperature of about 18-23°C.

19. The lyophilizate of claim 13, wherein the sugar comprises at least one member selected from the group consisting of a monosaccharide, a disaccharide and a trisaccharide.

20. The lyophilizate of claim 13, wherein the sugar comprises at least one member selected from the group consisting of glucose, mannose, galactose, fructose, sorbose, sucrose, lactose, maltose, cellobiose, gentiobiose, isomaltose, trehalose and raffinose.

21. The lyophilizate of claim 20, wherein the sugar comprises at least one member selected from the group consisting of sucrose, lactose, maltose, cellobiose, gentiobiose, isomaltose, trehalose and raffinose.

22. The lyophilizate of claim 13, wherein the amino sugar comprises at least one member selected from the group consisting of glucosamine, N-methyl-glucosamine, galactosamine and neuraminic acid.

23. The lyophilizate of claim 13, wherein the amino acid comprises at least one member selected from the group consisting of arginine, lysine, histidine, ornithine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine and tryptophan.

24. The lyophilizate of claim 13, wherein the surfactant comprises a polysorbate or a polyoxyethylene-polyoxypropylene polymer.

25. The lyophilizate of claim 13, wherein the monoclonal antibody or the polyclonal antibody has a molecular weight of 50-200 kDa per monomer unit.

26. The lyophilizate of claim 13, wherein the monoclonal antibody or the polyclonal antibody is directed against an antigen selected from the group consisting of hepatitis B virus, AIDS virus, cytomegalovirus, meningoencephalitis virus, rubella virus, measels virus, rabies pathogen, *Pseudomonas aeruginosa*, varicella-zoster virus, tetanus pathogen, van Willebrandt factor, nerve growth factor receptor, platelet derived growth factor receptor, selectin, integrin and diphtheria pathogen.

27. A lyophilizate, consisting essentially of

(1) a monoclonal antibody or a polyclonal antibody;

(2) a sugar or an amino sugar;

(3) at least one amino acid;

(4) a surfactant; and

(5) an inorganic acid as a buffering agent.

28. A liquid pharmaceutical composition comprising the lyophilizate of claim 13 dissolved in a physiologically acceptable solution.

29. The liquid pharmaceutical composition of claim 28, wherein the composition has a pH value of 5-8.

30. The liquid pharmaceutical composition of claim 28, wherein the composition contains 1-10 mg/ml of antibody.

31. The liquid pharmaceutical composition of claim 28, wherein the composition contains up to 200 mg/ml of sugar or amino sugar.

32. The liquid pharmaceutical composition of claim 28, wherein the composition contains up to 100 mg/ml of amino acid.

33. The liquid pharmaceutical composition of claim 28, wherein the composition contains 0.05-0.5 mg/ml of surfactant.

34. A liquid pharmaceutical composition comprising the lyophilizate of claim 27 dissolved in a physiologically acceptable solution.

35. The liquid pharmaceutical composition of claim 30, wherein the composition has a pH value of 5-8.

36. A method of preparing a lyophilizate, the method comprising

mixing a buffered solution containing a monoclonal antibody or a polyclonal antibody, a sugar or an amino sugar, at least one amino acid and a surfactant, to prepare a mixed solution, wherein the mixed solution has a pH value of 5-8; and

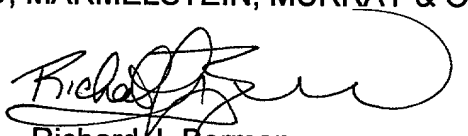
lyophilizing the mixed solution.--

REMARKS

The above amendment to the claims has been made to correct the multiple dependency of the claims and to put the application in better condition for examination.

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 14-1060.

Respectfully submitted,
NIKAIDO, MARMELSTEIN, MURRAY & ORAM LLP



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Stable lyophilized pharmaceutical preparations of monoclonal or polyclonal antibodies

The invention concerns lyophilized pharmaceutical preparations of monoclonal or polyclonal antibodies which contain a sugar or amino sugar, an amino acid and a surfactant as stabilizer. In addition the invention concerns a process for the production of these stable lyophilisates as well as the use of a sugar or amino sugar, an amino acid and a surfactant as stabilizers of therapeutic or diagnostic agents containing antibodies.

The production of immunoglobulins in particular monoclonal and polyclonal antibodies, for therapeutic and diagnostic purposes is nowadays of major and continuously increasing importance.

The use of antibodies as pharmacological agents has been already known for a long time and comprises numerous applications. Hence antibodies have been for example used successfully for tetanus prophylaxis, to combat pathogenic microorganisms or to neutralize their toxins and also for poisoning by snake venoms.

If the antigen involved in the disease mechanism has been identified, which is the case for numerous infectious and some oncological indications for antibody therapy, one utilizes the specificity of the antibodies for the therapy.

In clinical and preclinical studies antibodies are presently used to lower the cholesterol level, to influence the angiotensin/renin system and in autoimmune diseases such as for example lupus, autoimmune encephalitis, multiple sclerosis, polyarthritis and autoimmune myasthenia gravis.

Additionally of major therapeutic importance is their application to counteract intoxications by low molecular substances such as e.g. the Fab fragments of anti-digoxin antibodies when used for intoxications by digoxin or the cardiac glycosides digitoxin and ouabain. Moreover antibodies are used in the diagnostic field to identify, purify and determine the content of proteins.

Genetic engineering which revolutionized the production of monoclonal antibodies in cell cultures in the second half of the 70's and in the 80's has greatly advanced the preparation of antibodies.

In order to fulfil these diverse applications it is necessary to have pharmaceutical preparations of monoclonal and polyclonal antibodies that are stable on storage. There are a number of publications relating to liquid formulations or lyophilisates of special antibodies. Thus for example liquid formulations of antibodies are described in EP 0 280 358, EP 0 170 983, WO 89/11298, EP 0 352 500 and JP 63088197.

According to EP 0 280 358 dextran is added to the antibody solution to stabilize it towards certain hormones by which means it was possible to achieve a stability of over nine months. According to EP 0 170 983 hydrolysed ovalbumin is added to stabilize a

thermolabile monoclonal antibody when heated and as a result the antibody could still be used after storage at 45°C for 7 days. Polyhydroxy alcohols (e.g. glycerol, inositol, polyvinyl alcohol) or sugars (e.g. sucrose and glucose) or glycitols (e.g. sorbitol, mannitol) are known from JP 63088197 as further stabilizers for liquid formulations. WO 89/11298 demonstrates the use of maltose in a phosphate buffer containing sodium chloride as a further method for the liquid stabilization of monoclonal antibodies. EP 0 352 500 describes polyethylene glycol 4000 and 3-propiolactone for the liquid stabilization of monoclonal antibodies.

However, in general liquid formulations are not an optimal solution due to storage stability since the proteins or aggregates thereof may precipitate in time during storage, at increased temperatures, when transported through different climatic zones or by improper storage (e.g. interruptions in the cool chain) and the solutions may thus have a reduced protein content and become turbid. Hence, a problem-free use of the solutions cannot be guaranteed in these cases.

In contrast in the case of a lyophilisate formulation the removal of water minimizes the formation of degradation products (e.g. by deamidation and hydrolysis) and aggregate formation. The residual content of water (bound water) can contribute to the stability particularly in the presence of sugars (Hsu et al. Dev. Biol. Stand. 1991, 74: 255-267 and Pikal et al., Dev. Biol. Stand. 1991, 74: 21-27).

Lyophilisate formulations with special antibodies as active substances are also known from the literature but

they do not give consistent advice about the problem of stabilization. Hence in WO 93/00807 the stabilization of biomaterials is described such as human proteins, growth hormones, interleukins, interferons, enzymes and also monoclonal and polyclonal antibodies by a two component system consisting of cryoprotective agents (e.g. polyethylene glycols) and a compound which can form hydrogen bridges with proteins. However, a disadvantage of these preparations is that the addition of high molecular compounds such as polyethylene glycols can lead to an accumulation in the body with potentially toxic side-effects if there is no biodegradation. Furthermore, as is well-known, polymers can act as antigens depending on their molar mass.

Lyophilisates of a monoclonal antibody that is labile when frozen are stabilized for one year according to JP 60146833 by the addition of albumin (human, horse or bovine albumin). Human serum albumin (HSA) is also described in EP 0 303 088 in combination with a carbohydrate (e.g. dextrose, sucrose or maltose) to stabilize a monoclonal antibody for the treatment of Pseudomonas aeruginosa infections.

Human serum albumin (in combination with sugars and amino acids) is also the principle by which monoclonal antibodies are stabilized in EP 0 413 188. In JP 01075433 a mixture of human serum albumin, mannitol and polyethylene glycol is used to stabilize a human monoclonal antibody as a lyophilisate. A further example of the use of macromolecules such as e.g. polyethylene glycols and protecting proteins such as human serum albumins to stabilize gamma-globulins during lyophilization is shown in WO 84/00890.

In WO 93/01835 Hagiwara et al. describe the stabilization of a human monoclonal antibody by lyophilization with mannitol and glycine in a solution containing sodium chloride and phosphate buffer. Stable preparations are obtained with regard to freezing, lyophilization and reconstitution.

Draber et al. (J. Immun. Methods, 1995, 181:37-43) were able to produce a stable formulation of monoclonal IgM antibodies from the mouse at 4°C by the addition of trehalose alone and in combination with polyethylene glycol 8,000. However, the antibodies are only stable for 14 days at 50°C. Using other monosaccharides or disaccharides alone such as e.g. sucrose, maltose, lactose or galactose it is not possible to stabilize these antibodies.

A monoclonal antibody from the mouse is converted into a stable lyophilisate in WO 89/11297 using a carbohydrate (maltose) and a buffer in the acid range (acetate buffer). In this case a disadvantage is the limitation to buffering in an acid range.

Polymeric gelatin as a freezing protectant and stabilizer in a lyophilisate is used in WO 92/15 331. The stabilization is also achieved in combination with a carboxylic acid (e.g. citric acid) or a salt thereof as well as with a primary, secondary or tertiary alcohol or an amino acid in a pH range of 6.8 to 8.1.

In a whole series of the aforementioned publications pharmaceutical additives or auxiliary substances are proposed as stabilizers which are not acceptable from a medical point of view. Hence polymers (such as PEG or

gelatin) and proteins (such as serum albumins) pose a certain risk due to their origin and their physico-chemical properties and can trigger allergic reactions even to the point of an anaphylactic shock. Proteins of human or animal origin as well as proteins obtained from cell cultures carry the residual risk of viral contaminations. However, other protein-like contaminations which are difficult to detect analytically can cause immunological reactions in humans due to their properties.

The addition of polymeric compounds such as e.g. polyethylene glycols (PEG) or gelatin can lead to an accumulation in the body with potentially toxic side-effects if there is no biodegradation. Polymers may also have antigenic properties depending on their molar mass. Also it is difficult to ensure the purity of polymers due to the catalysts used in their production or the presence of monomers and other polymer fragments. The use of polymers in pharmaceutical forms of administration, especially in drug forms that can be administered subcutaneously, should be avoided if another type of stabilization is possible.

In contrast the use of sugars alone without other additives does not always ensure an adequate protective effect when the antibodies are lyophilized.

Hence the object of the invention was to provide a stable pharmaceutical preparation of monoclonal or polyclonal antibodies that is essentially free of the above-mentioned polymers or proteinaceous pharmaceutical auxiliary substances. This applies particularly to those antibodies which are labile towards freezing and thawing

processes or towards multiple freezing and thawing processes.

Surprisingly it was found that stable pharmaceutical lyophilisates of monoclonal or polyclonal antibodies are obtained if these contain sugar or amino sugar, an amino acid and a surfactant as additives. The lyophilisates are preferably composed of a) the antibody, b) a sugar or amino sugar, c) an amino acid, d) a buffer for adjusting the pH value and e) a surfactant. Those lyophilisates are particularly preferred which only contain a single or two different amino acids.

These preparations are physiologically well tolerated, have a relatively simple composition and can be dosed exactly. In addition they are stable i.e. they exhibit no detectable degradation products or protein aggregates when subjected to multiple freezing and thawing processes as well as on longer storage. The lyophilisates can even be stored without stability problems at refrigerator temperature (4 - 12°C) or even at room temperature (18 - 23°C) over a time period of at least three months, preferably at least six months and in particular of at least one to two years. Furthermore they are also stable when stored at higher temperatures (for example up to 30°C). The storage stability is for example exhibited by the fact that during the said storage period only a very small number of particles can be detected when the lyophilisates are reconstituted in the containers with water for injection purposes or with isotonic solutions. In particular the containers have fewer than 6000 particles with a particle size of more than 10 µm and/or less than 600 particles with a particle size of more than 25 µm. The solutions prepared in this manner are stable over a time period of about up

to five days, preferably up to three days.

The fact that the preparations protect against freezing due to the selected combination of additives is particularly advantageous. Hence, in particular this enables a lyophilization at temperatures down to -45°C without impairing the stability of the antibodies. In addition the lyophilisates containing the combination of additives according to the invention are also stable for a long period and during storage even at relatively high temperatures. Especially compared to conventional formulations, they exhibit no particle formation after reconstitution with water, i.e. the solutions are essentially free of turbidities.

The preparations according to the invention have the additional advantage of being essentially free of protein-like or polymeric auxiliary substances the use of which may be problematic from a medical point of view. Due to the fact that liquid therapeutic or diagnostic agents containing antibodies with a pH value of about 5 to 8, preferably with a pH value of 6.0 - 7.4 (pH value of blood 7.2 - 7.4) can now be prepared by dissolving lyophilisates, they have the additional advantage of being well-tolerated and can be administered substantially free of pain. This is above all important for subcutaneous administration since in this case intolerances develop more easily than when administered intravenously.

The formulations according to the invention can in general be produced in clinically relevant concentration ranges of the antibody for example of up to 20 mg/ml preferably up to 10 mg/ml. Preferred concentration

ranges are concentrations above 0.01 mg/ml in particular above 0.05 and 0.1 mg/ml. In particular concentration ranges of 0.05 - 10 mg/ml or 0.1 - 5 mg/ml for example about 5, 8 or 10 mg/ml are used. The injection volumes of the solutions used are less than 2 ml preferably about 1 ml in the case of subcutaneous or intravenous injections. Small injection volumes are particularly advantageous for subcutaneous administration since they only cause slight mechanical irritation in the subcutaneous tissue. Basically the solutions are also directly suitable as additives to infusion solutions or as infusion solutions. If they are used as additives to infusion solutions the concentration of the antibodies is at higher levels, for example up to 10 mg/ml. These concentrated solutions of the antibodies are then added to conventional infusion solutions so that the concentration of the antibody in the infusion solution to be administered is in the therapeutically relevant range. This range is normally 0.001 - 0.5 mg/ml.

The pharmaceutical single forms of administration can either be present as ready-to-use infusion solutions or injection solutions or also as lyophilisates. If the pharmaceutical preparations are used in the form of lyophilisates, the single dose containers, for example glass ampoules with a volume of 10 ml, contain the antibody in amounts of 0.1 - 500 mg, preferably 10 - 100 mg depending on the respective therapeutically relevant dose of the antibody. The lyophilisate optionally contains additional conventional pharmaceutical auxiliary substances. The lyophilisate is dissolved with an appropriate amount of reconstitution solution and can then either be used directly as an injection solution or as an additive to an infusion solution. If it is used as an additive to infusion

solutions, the lyophilisate is usually dissolved with about 10 ml of a reconstitution solution and added to a physiological saline solution (0.9 % NaCl) of 250 ml. The resulting infusion solution is then usually administered to the patient within about 30 minutes.

The sugars used according to the invention can be monosaccharides, disaccharides or trisaccharides. Glucose, mannose, galactose, fructose and sorbose come into consideration as monosaccharides. Sucrose, lactose, maltose or trehalose come into consideration as disaccharides. Raffinose is preferably used as the trisaccharide. According to the invention sucrose, lactose, maltose, raffinose or trehalose are especially preferably used. Instead of maltose it is also possible to use the stereoisomeric disaccharides cellobiose, gentiobiose or isomaltose.

Those monosaccharides are generally referred to and used as amino sugars which have an amino ($-NH_2$, -NHR, $-NR_2$) or an acylated amino group (-NH-CO-R) instead of a hydroxy group. For this glucosamine, N-methyl-glucosamine, galactosamine and neuraminic acid are particularly preferred according to the invention. The sugar content or amino sugar content is for example up to 2000 mg, preferably up to 1000 mg especially up to 800 or up to 500 mg per single form of administration. Amounts of more than 10, 50 or 100 mg come for example into consideration as the lower limit for the sugar content. Preferred ranges are 200 - 1000 mg, especially 400 - 800 mg. The stated quantities per single form of administration refer to single forms of administration which are marketed as lyophilisates. Such lyophilisates are preferably filled into injection bottles with a volume of 10 ml. After dissolution of the lyophilisates

with a reconstitution solution of 10 ml, liquid forms of administration are obtained which can be administered directly. The sugar concentration in these injection solutions is up to 200 mg/ml, preferably up to 100 mg/ml based on the amounts stated above of the sugars used.

The amino acids used according to the invention can be basic amino acids such as arginine, lysine, histidine, ornithine etc., the amino acids preferably being used in the form of inorganic salts thereof (preferably in the form of phosphoric acid salts i.e. as amino acid phosphates). If free amino acids are used, the desired pH value is adjusted by adding a suitable physiologically tolerated buffer substance such as e.g. an inorganic acid in particular phosporic acid, sulphuric acid, acetic acid, formic acid or salts thereof. In this case the use of phosphates has the particular advantage that particularly stable lyophilisates are obtained. It has proven to be advantageous when the preparations are essentially free of organic acids such as e.g. malic acid, tartaric acid, citric acid, succinic acid, fumaric acid, etc. or the corresponding anions (malates, oxalates, citrates, succinates, fumarates, etc.) are not present.

Preferred amino acids are arginine, lysine or ornithine. In addition it is also possible to use acidic amino acids such as glutamic acid and aspartic acid or neutral amino acids such as e.g. isoleucine, leucine and alanine or aromatic amino acids such as e.g. phenylalanine, tyrosine or tryptophan. The amino acid content in the aqueous preparations according to the invention is up to 100 mg/ml, preferably up to 50 mg/ml or up to 30 mg/ml. The lower limit may for example be concentrations above 1, 5 or 10 mg/ml. Preferred concentrations are for example in the range of 3 - 30 mg/ml or 10 - 25 mg/ml.

If the corresponding forms of administration are marketed as lyophilisates, these lyophilisates are preferably made available in injection bottles (volumes of for example 10 ml). Such single forms of administration contain the amino acids in amounts of up to 1000 mg, preferably up to 500 mg or up to 300 mg.

Surfactants which come into consideration are all surfactants that are usually used in pharmaceutical preparations preferably polysorbates and polyoxy-ethylene-polyoxypropylene polymers such as e.g. Tween®. Low amounts of surfactant of 0.05 to 0.5 mg/ml preferably 0.1 mg/ml are sufficient to stabilize the antibodies. In the above-mentioned single forms of administration the amount of surfactants is 0.5 - 5 mg in the case of a lyophilisate that is filled into an injection bottle of 10 ml.

The stabilization of antibodies achieved by the said additives relates in principle to all known monoclonal and polyclonal antibodies and their Fab fragments. Humanized antibodies and modified antibodies (cf. e.g. US 5,624,821; EP 0 592 106; PCT/EP96/00098) are preferably used. The molecular weight of the antibodies is 50 kDa-200 kDa per monomer unit, in particular the molecular weight is about 80 - 150 kDa. In particular antibodies to the hepatitis B virus (cf. WO 94/11495), to AIDS viruses, cytomegalo viruses, meningoencephalitis viruses (FSME), rubella viruses, measles viruses, rabies pathogens, Pseudomonas aeruginosa bacteria, varicella-zoster viruses, tetanus pathogens, van Willebrandt factor (cf. WO 96/17078), NGFR (nerve growth factor receptor), PDGFR (platelet derived growth factor receptor: Shulman, Sauer, Jackman, Chang, Landolfi, J. Biol. Chem. 1997, 272(28): 17400-4), selectin, in particular E-selectin,

L-selectin (cf. Takashi et al., Proc. Natl. Acad. Sci. USA 1990, 87: 2244-2248; WO 94/12215) or P-selectin; integrins or diphtheria pathogens can be stabilized according to the invention. The antibody concentration can preferably be up to 8 mg/ml. It is preferably for example 0.05 - 2 mg/ml. The amount of antibody in the single form of administration, for example in a lyophilisate in an injection bottle of 10 ml, is up to 100 mg preferably up to 80 mg, 50 mg, 20 mg or 10 mg. The concentration of the antibodies after reconstitution of the lyophilisates with a volume of 10 ml is in the range of 1 - 10 mg/ml, preferably at 5 - 8 mg/ml.

In addition to the said additives, sugar, amino acid and surfactant, the lyophilisates according to the invention can contain physiologically tolerated auxiliary substances from the group comprising acids, bases, buffers or isotonizing agents to adjust the pH value to 5 to 8, preferably 6.0 to 7.4. The buffer capacity of the preparations is adjusted such that when the lyophilisates are dissolved with standard reconstitution solutions such as for example water for injection purposes the buffer concentration is in the range between 10 - 20 mmol/l preferably at about 15 mmol/l.

The order of addition of the various auxiliary substances or of the antibody is largely independent of the production process and is up to the judgement of a person skilled in the art. The desired pH value of the solution is adjusted by adding bases such as for example alkali hydroxides, alkaline earth hydroxides or ammonium hydroxide. Sodium hydroxide is preferably used for this. The desired pH value can in principle be adjusted by adding basic solutions. In this sense salts of strong bases with weak acids are generally suitable such as

sodium acetate, sodium citrate, di-sodium or sodium dihydrogen phosphate or sodium carbonate. If the pharmaceutical solution of auxiliary substances has a basic pH value it is adjusted by titration with an acid until the desired pH range has been reached. Physiologically tolerated inorganic or organic acids come into consideration as acids such as for example hydrochloric acid, phosphoric acid, acetic acid, citric acid or conventional solutions of substances which have an acidic pH value. In this sense preferred substances are salts of strong acids with weak bases such as e.g. sodium dihydrogen phosphate or disodium hydrogen phosphate. The pH value of the solution is preferably adjusted with phosphoric acid or an aqueous sodium hydroxide solution.

In order to produce well-tolerated parenteral drug forms it is expedient to add isotonizing auxiliary substances if isotonicity cannot be already achieved by the osmotic properties of the antibody and the additives used for stabilization. Non-ionized well-tolerated auxiliary substances are used above all for this. Salts such as NaCl should, however, only be added in small amounts, in particular a value of 30 mmol/l in the final injection or infusion solution for administration should not be exceeded.

In addition the pharmaceutical preparations can contain further common auxiliary substances or additives. Antioxidants such as for example glutathione or ascorbic acid or similar substances can be added.

For the production of the lyophilisates the aqueous pharmaceutical solutions which contain the antibody are

firstly produced. A buffered antibody solution containing sodium chloride is preferably prepared. This antibody solution is admixed with an aqueous solution containing the additives sugar, amino acid and surfactant during which the pH value is adjusted with an acid or base to 5 to 8. Phosphoric acid or phosphate salts and sodium chloride are added in such amounts that the previously defined concentrations are obtained. Subsequently it is sterilized by filtration and the solution prepared in this manner is lyophilized.

The invention also enables unstable aqueous solutions containing antibodies that are sensitive to freezing to be also converted by means of freeze-drying into stable preparations that are also stable at high temperatures without impairing the quality.

A further advantage of the lyophilisates according to the invention is that, in addition to avoiding damage to the antibodies during freezing, they also exhibit no reduction in the antibody content and no aggregate formation or flocculation even after a long-term storage at 50°C. They are thus stable with regard to antibody content and purity. The formation of particles is prevented which is exhibited by the low values for turbidity after reconstitution of the lyophilisates with water for injection purposes.

The invention is elucidated in more detail in the following on the basis of examples of application.

Examples 1 to 10 show in which manner the lyophilisates according to the invention can be formulated, produced and examined with regard to antibody stability.

Comparative experiments without auxiliary substances or with sucrose alone or with mannitol as a substitute for the sugar component or with the amino acid component alone or only the sugar or amino acid component without the surfactant show that the choice of the combination of additives according to the invention is essential for achieving a stable formulation. Sucrose alone, amino acid alone or both components without surfactant lead to unstable formulations.

The formulations according to the invention are insensitive to freezing and it is possible to completely omit polymers or proteins that are regarded as being toxic such as polyethylene glycols, gelatin, serum albumins. In the case of the surfactants only relatively small amounts of physiologically well-tolerated surfactants are present.

The antibody to HBV used in the following application examples is a recombinant human monoclonal antibody (MAB) from a murine cell. It has a molecular weight of about 147 kDa and is directed towards the hepatitis B surface antigen (HBsAg) of the hepatitis B virus. The monoclonal antibody recognizes the a-determinant of the HBsAg which is constant in almost all known variants of the virus. This antibody can for example be used for the following medical indications: treatment of chronic hepatitis for which there has previously not yet been a satisfactory treatment method; treatment of passive immunoprophylaxis in HBsAg-positive liver transplant patients. In central and northern Europe and the USA up to 2 % of the population are carriers of the hepatitis B virus, in southern Europe up to 3 %, in Africa and the Far East it is 10 - 15 %. A consequence of this chronic infection is that the risk of developing hepatocellular

carcinoma is increased by 100-fold, 40 % of the virus carriers die as a result of this infection.

Antibodies to L-selectin, the NGF receptor or the PDGF receptor can be preferably used as antibodies within the sense of the invention.

Example 1 shows the properties of an aqueous solution of a monoclonal antibody to hepatitis B virus (MAB HBV; INN name: Tuvirumab) containing phosphate buffer and sodium chloride at pH = 5, pH = 6.5 and pH = 8 after freezing and thawing. It shows that freezing and thawing damages the monoclonal antibody.

Example 2 demonstrates the possibility of stabilizing a preparation according to the invention with sucrose or maltose or an amino sugar (N-methylglucosamine or galactosamine) and arginine phosphate and Tween 20 with a concentration of the antibody of 2 mg/ml i.e. 2 mg in the lyophilisate.

The same preparation as in example 2 is shown in example 2a except that the antibody concentration is 8 mg/ml. It can be seen from examples 2 and 2a that the combination of the said auxiliary substances not only avoids damage to the antibody during freezing but also has a positive influence on the stability during long-term storage.

Example 3 elucidates the necessity of amino acids and surfactant in the preparation according to the invention. The use of sucrose as a builder alone leads to an unstable lyophilisate.

Example 4 describes variations of the amino acid component. It turns out that variation of the basic amino acids in the form of arginine or ornithine as well as the substitution of the basic amino acid by a neutral amino acid such as e.g. by leucine or by an acidic amino acid such as e.g. aspartic acid leads to a storage-stable preparation.

In example 5 the lyophilisation of a formulation containing sucrose, arginine and Tween 20 as well as phosphate buffer and sodium chloride is compared at various pH values (pH 5, pH 6.5 and pH 8). The data obtained show that it is possible to lyophilize within this pH range without impairing the stability.

If the said surfactant Tween 20 is replaced by a representative of the surface-active class of compounds polyoxyethylene-polyoxypropylene polymers (commercial name Pluronic®) as in example 6 this also results in an adequate stability of the preparation according to the invention.

Example 7 demonstrates the instability of a formulation containing mannitol as the builder as a substitute for sucrose, maltose or the amino sugar (see example 2).

If the sugar and the surfactant are omitted in the formulation the preparation becomes unstable as shown in example 8.

Although a combination of sugar (e.g. sucrose) and amino acids without surfactant in example 9 yields good results with regard to the parameters content and aggregates, the turbidity is, however, substantially

increased compared to the formulations according to the invention containing sugar, amino acid and surfactant.

Example 10 shows that other monoclonal antibodies can also be stabilized with a combination of the sugar, amino acid and surfactant. The antibody anti-L-selectin is for example stable at a concentration of 7 mg in the lyophilisate. The lyophilization is carried out starting with a volume of 1 ml of an aqueous solution.

Investigative methods to determine stability

The lyophilized preparations were stored under defined storage conditions in the absence of light and subsequently analysed. The following test methods were used for the analyses.

OD280: Optical density at 280 nm. Photometric determination of the protein content, the UV absorbance is due to side chain chromophores such as tryptophan tyrosine and phenylalanine residues. Specification: 95-105 %.

SE-HPLC: Size-exclusion high performance chromatography to determine aggregates. Specification: max. 2 %.

Measurement of turbidity: After reconstituting the lyophilisate the undiluted antibody solution was measured in a suitable turbidity photometer. Specification: max. 6 turbidity units.

Example 1:

An aqueous stock solution of the MAB to HBV described above containing phosphate buffer and sodium chloride is prepared and examined. The concentration of the MAB is about 15 mg/ml.

Table 1a shows on the one hand the lability to freezing of the monoclonal antibody solution at various pH values at -20°C which already results in a decrease of the protein content after 4 weeks to 92.1 and 94.2 and 94.0 %. A decrease of the protein content is also observed on storage at 25°C. Under the storage conditions 4-8°C in a refrigerator the antibody is adequately stable over 9 months.

Tables 1b-1d show the stability data of the monoclonal antibody solution prepared at pH values 5, 6.5 and 8 at -20°C, 4-8°C and 25°C. This also shows that only a storage at 4-8°C is acceptable.

Table 1a: Change of the antibody content in the solution of active substance (10 mM phosphate buffer, 30 mM sodium chloride, water for injection purposes)

	pH 5			pH 6.5			pH 8		
Time	-20°C	4-8°C	25°C	-20°C	4-8°C	25°C	-20°C	4-8°C	25°C
start		> 99			> 99			> 99	
4 weeks	92.1	> 99	> 99	94.2	> 99	> 99	94.0	> 99	> 99
13 weeks	78.9	> 99	97.2	81.2	> 99	98.1	77.8	> 99	96.1
6 months	61.2	> 99	94.1	69.9	> 99	94.4	65.8	> 99	91.9
9 months	47.8	> 99	88.7	55.6	> 99	90.2	51.0	> 99	84.3

All data in %. The protein was determined by measuring the absorbance at 280 nm (OD 280).

Table 1b: Aggregate formation and turbidity values for the active substance solution of antibody, pH = 5

Times	-20°C		4-8°C		25°C	
	aggregates	turbidity	aggregates	turbidity	aggregates	turbidity
start	n.d.	1.5	n.d.	1.5	n.d.	1.5
4 weeks	aggregates	floccul.	n.d.	1.5	0.7 %	1.5
13 weeks	aggregates	floccul.	0.2 %	1.8	1.9 %	1.8
6 months	aggregates	floccul.	0.3 %	1.9	aggregates	9.9
9 months	aggregates	floccul.	0.6 %	2.1	aggregates	10.9

n.d.= not detectable

Table 1c): Aggregate formation and turbidity values for the active substance solution of antibody, pH = 6.5

Times	-20°C aggregates	turbidity	4-8°C aggregates	turbidity	25°C aggregates	turbidity
start	n.d.	1.2	n.d.	1.2	n.d.	1.2
4 weeks	aggregates	floccul.	n.d.	1.3	0.5 %	1.4
13 weeks	aggregates	floccul.	0.2 %	1.4	1.8 %	1.7
6 months	aggregates	floccul.	0.3 %	1.9	4.9 %	floccul.
9 months	aggregates	floccul.	0.6 %	2.1	9.3 %	floccul.

Table 1d: Aggregate formation and turbidity values for the active substance solution of antibody, pH = 8

Times	-20°C aggregates	turbidity	4-8°C aggregates	turbidity	25°C aggregates	turbidity
start	n.d.	1.4	n.d.	1.4	n.d.	1.4
4 weeks	2.0 %	floccul.	0.3 %	1.5	0.74 %	1.7
13 weeks	2.8 %	floccul.	0.5 %	1.8	1.95 %	2.1
6 months	3.7 %	floccul.	0.6 %	1.9	3.0 %	floccul.
9 months	5.4 %	floccul.	0.8 %	2.1	4.3 %	floccul.

Aggregates in % using SE-HPLC, turbidity in turbidity units (turbidity) using a turbidity photometer.

Example 2:

A solution of the monoclonal antibody to HBV according to example 1 was added to aqueous solutions of the

following sugars or amino sugars: sucrose (formulation 1), maltose (formulation 2) and N-methylglucosamine (formulation 3) containing arginine phosphate buffer and Tween 20 as the surfactant. The formulation is listed in example 2a. The final concentration of the MAB is 2 mg/ml. After adjusting the pH value with phosphoric acid to 6.5, the solutions were sterilized by filtration (0.22 µm membrane filter) and filled into sterilized and depyrogenized injection bottles made of glass (hydrolytic class I) (filling volumes 1 ml) and lyophilized. After lyophilization the injection bottles were aerated with nitrogen, sealed automatically with stoppers in the freeze drying chamber and subsequently flanged.

The flanged injection bottles were stored in the absence of light for 4 to 13 weeks at various temperatures. After these periods the stability of the lyophilisates was examined with the described methods of examination.

Table 2a: Storage at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form. 1 sucrose	100	n.d.	1.7	100	n.d.	1.6
form. 2 maltose	100	n.d.	1.6	100	n.d.	1.8
form. 3 N-methyl-glucosamine	100	n.d.	1.8	100	n.d.	1.5

Table 2b: Storage at 50°C

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form. 1 sucrose	>99	n.d.	2.0	>99	n.d.	2.0
form. 2 maltose	>99	n.d.	1.9	>99	n.d.	2.1
form. 3 N-methyl-glucosamine	>99	n.d.	1.7	>99	n.d.	2.0

Legend:

I protein content in % with OD 280

II aggregates in % with SE-HPLC

III turbidity of the reconstituted solution in turbidity units (dimensionless number)

n.d. not detectable (used in the same way in all further tables)

Example 2a

In example 2a the formulation 1 from example 2 is prepared with 8 mg/ml antibody (= formulation 1a). It turns out that higher concentrations of up to 8 mg/1 ml antibody are adequately stable in this formulation.

Compositions of formulations 1 and 1a:

	Formulation 1	Formulation 1a
MAB HBV	2.0 mg	8.0 mg
phosphate buffer	15 mM	15 mM
sodium chloride	30 mM	30 mM
sucrose	68.0 mg	58.0 mg
arginine	10.0 mg	10.0 mg
phosphoric acid	ad pH 6.5	ad pH 6.5
Tween 20	0.1 mg	0.1 mg
water for injection purposes	ad 1.0 ml	ad 1.0 ml

Table 3a: Stability data for formulation 1 and formulation 1a at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form.1: 2 mg/1ml	100	n.d.	1.7	100	n.d.	1.6
form.1a:8 mg/1ml	>99	n.d.	4.8	>99	n.d.	4.7

Table 3b: Stability data for formulations 1 and 1a at 50°C

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form.1: 2 mg/1ml	>99	n.d.	2.0	>99	n.d.	2.0
form.1a:8 mg/1ml	>99	n.d.	4.7	>99	n.d.	5.5

I protein content in % with OD 280

II aggregates in % with SE-HPLC

III turbidity of the reconstituted solution in turbidity units (dimensionless number)

Example 3

Comparison of formulations 1 and 4. Formulation 4 only contains sucrose as the builder and no arginine phosphate and no Tween 20. Formulation 4 is unstable.

	Formulation 1	Formulation 4
MAB HBV	2.0 mg	2.0 mg
phosphate buffer	15 mM	15 mM
sodium chloride	30 mM	30 mM
sucrose	68.0 mg	68.0 mg
arginine	10.0 mg	--
phosphoric acid or NaOH	ad pH 6.5	ad pH 6.5
Tween 20	0.1 mg	--
water for injection purposes	ad 1.0 ml	ad 1.0 ml

Table 4a: Storage at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form.1: sucrose with arg. phos. and Tween 20	100	n.d.	1.7	>99	n.d.	1.6
form.4: sucrose without arg.phos. and Tween 20	98.3	1.6	6.1	96.0	4.3	9.5

Table 4b: Storage at 50°C:

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form.1:sucrose with arg.phos. and Tween 20	100	n.d.	2.0	>99	n.d.	2.0
form.4:sucrose without arg.phos. and Tween 20	96.0	4.2	8.5	89.8	10.1	10.9

Legend:

I protein content in % with OD 280

II aggregates in % with SE-HPLC turbidity of the reconstituted solution in turbidity units (dimensionless number)

III turbidity of the reconstituted solution in turbidity units (dimensionless number)

Example 4

Variation of the amino acid component of the formulation. Formulations with basic, acidic and neutral amino acids are stable.

Composition of the formulations:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	35 - 70 mg
amino acid	variable
phosphoric acid or NaOH	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

Table 5

	Amino acid
formulation 1	arginine (basic)
formulation 5	ornithine (basic)
formulation 6	leucine (neutral)
formulation 7	aspartic acid (acidic)

The pH value is adjusted by phosphoric acid or hydroxide solution.

Tables 6a - d

Examination results of formulations 1, 5, 6, 7 after storage for 4 and 13 weeks.

a) Table 6a, Formulation 1 (arginine):

	Storage period 4 weeks 25°C	50°C	Storage period 13 weeks 25°C	50°C
protein content % (OD 280)	100	>99	100	>99
aggregates % (SE-HPLC)	n.d.	n.d.	n.d.	n.d.
turbidity	1.7	2.0	1.6	2.0

b) Table 6b, formulation 5 (ornithine):

	Storage period 4 weeks 25°C	50°C	Storage period 13 weeks 25°C	50°C
protein content % (OD 280)	>99	>98	>98	>98
aggregates % (SE-HPLC)	n.d.	n.d.	n.d.	n.d.
turbidity	1.9	1.9	2.0	2.1

c) Table 6c, formulation 6 (leucine):

	Storage period 4 weeks 25°C	50°C	Storage period 13 weeks 25°C	50°C
protein content % (OD 280)	>98	>98	>98	>98
aggregates % (SE-HPLC)	n.d.	n.d.	0.1	0.1
turbidity	2.2	2.4	2.8	2.7

d) Table 6d, formulation 7 (aspartic acid):

	Storage period 4 weeks 25°C	50°C	Storage period 13 weeks 25°C	50°C
protein content % (OD 280)	>98	>98	>98	>98
aggregates % (SE-HPLC)	n.d.	n.d.	0.1	0.1
turbidity	2.7	2.7	3.4	4.0

Example 5

Example 5 contains formulation 1 at various pH values, the lyophilisates are prepared as described in example 2, the pH of the solution of auxiliary substances and of the product solution was adjusted before lyophilisation with 85 % phosphoric acid.

Formulation:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	68 mg
arginine	10 mg
phosphoric acid	ad pH 5; 6.5; 8
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

The lyophilisates were prepared with the pH values shown in table 7.

After flanging the injection bottles these were stored in the absence of light under defined temperature conditions. After storage periods of 4 weeks and 13 weeks the samples were analysed (protein content in %: OD 280, aggregates in %: SE-HPLC, turbidity). The formulation was stable at all pH values.

Table 7:

	pH
formulation 8	5
formulation 9 (ident. with 1)	6.5
formulation 10	8

Table 8a: Storage at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
formulation 8	100	n.d.	1.9	>99	n.d.	2.3
formulation 9(=1)	100	n.d.	1.7	100	n.d.	1.6
formulation 10	>99	n.d.	2.3	>99	n.d.	2.6

Table 8b: Storage at 50°C

	Storage 13 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
formulation 8	>99	n.d.	2.2	>99	n.d.	2.3
formulation 9(=1)	>99	n.d.	2.0	>99	n.d.	2.0
formulation 10	>98	n.d.	2.5	>98	n.d.	2.6

Legend:

I protein content in % with OD 280

II aggregates in % with SE-HPLC

III turbidity of the reconstituted solution in turbidity units (dimensionless number)

Example 6

The formulation described in the following containing the surfactant Pluronic F 68 instead of Tween 20 was prepared as described above.

The storage and examination of stability was carried out in an analogous manner to that of the other examples.

Formulation 11:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	48.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
Pluronic F 68	0.1 mg
water for injection purposes	ad 1.0 ml

Formulation 1 is chosen as a comparison and is identical to formulation 11 except for Tween 20 instead of Pluronic F 68. Both formulations were stable.

Table 9: Stability data of the formulation containing the surfactants Pluronic F 68 and Tween 20.

	formulation 11 storage period 4 weeks 25°C	50°C	storage period 13 weeks 25°C	50°C	formulation 1 storage period 4 weeks 25°C	50°C	storage period 13 weeks 25°C	50°C
protein content % (OD 280)	>98	>98	>98	>98	100	>99	100	>99
aggregates % (SE-HPLC)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
turbidity	1.9	1.9	2.5	2.2	1.7	2.0	1.6	2.0

Example 7:

The formulation 12 described in this example essentially corresponds to formulation 1 except that mannitol was used instead of sucrose as a builder. It can be seen that the mannitol formulation is unstable.

Formulation 12:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
mannitol	25.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

Table 10: Stability data of the formulations containing the builder mannitol (formulation 12) and sucrose (formulation 1)

	formulation 12 storage period				formulation 1 storage period			
	4 weeks		storage period 13 weeks		4 weeks		storage period 13 weeks	
	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
protein content % (OD 280)	96.2	91.8	94.0	84.5	100	>99	100	>99
aggregates % (SE-HPLC)	3.6	8.4	5.8	15.9	n.d.	n.d.	n.d.	n.d.
turbidity	3.2	6.9	4.9	13.2	1.7	2.0	1.6	2.0

Example 8

Further evidence for the necessity of the combination of sugar, amino acid and surfactant is given by comparing formulation 1 which contains all listed components with formulation 13 composed of antibody, phosphate buffer, sodium chloride and arginine phosphate. The aggregate formation is increased and the turbidity values become worse without sugar and surfactant.

Formulation 13

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
arginine	35.0 mg
phosphoric acid	ad pH 6.5
water for injection purposes	ad 1.0 ml

Table 11: Stability data for formulation 13 (without sucrose and Tween 20 only with arginine phosphate as builder) and formulation 1

	formulation 13 storage period 4 weeks 25°C	50°C	storage period 13 weeks 25°C	50°C	formulation 1 storage period 4 weeks 25°C	50°C	storage period 13 weeks 25°C	50°C
protein content % (OD 280)	97.6	94.9	95.8	89.0	100	>99	100	>99
aggregates % (SE-HPLC)	2.6	4.5	4.0	10.7	n.d.	n.d.	n.d.	n.d.
turbidity	2.9	4.5	3.8	12.3	1.7	2.0	1.6	2.0

Example 9

Although a stable formulation is obtained without surfactant (Tween 20) and only with sucrose and arginine, the turbidity values worse (formulation 14).

Formulation 15:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	68.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
water for injection purposes	ad 1.0 ml

Table 12: Stability data of formulation 14 and formulation 1

	formulation 14 storage period				formulation 1 storage period			
	4 weeks		storage period 13 weeks		4 weeks		storage period 13 weeks	
	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
protein content % (OD 280)	>99	>98	>98	>98	100	>99	100	>99
aggregates % (SE-HPLC)	0.2	0.3	0.5	1.3	n.d.	n.d.	n.d.	n.d.
turbidity	3.4	4.8	8.8	13.3	1.7	2.0	1.6	2.0

Example 10

The following table shows the components of formulation 15. The antibody used is anti-L-selectin. The data shown in table 13a on the examination of stability show that the formulation used enables an adequate stabilization.

Composition of formulation 15:

	Formulation 15
anti-L-selectin	7.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	68.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

Table 13a: Stability data for formulation 15 at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form. 15:7 mg/1ml	>99	n.d.	2.5	>99	n.d.	2.9

Table 13b: Stability data for formulation 15 at 50°C

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form. 15:7 mg/ml	99	n.d.	4.1	99	n.d.	5.2

I protein content in % with OD 280
II aggregates in % with SE-HPLC
III turbidity of the reconstituted solution in turbidity units (dimensionless number)

Example 11

Stabilization of the antibody anti-L-NGFR (anti-L-nerve-growth-factor-receptor)

Formulation 16:

A lyophilisate with the following formulation (analogous to formulation 1) is prepared:

	Formulation 16
anti-L-NGFR	0.25 mg
phosphate buffer	15 mM
sucrose	75 mg
arginine	10 mg
phosphoric acid	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

The lyophilisate of anti-L-NGFR is prepared analogously to the preparation of the MAB-HBV and anti-L-selectin lyophilisates.

An aqueous solution containing the additives sugar, amino acid and surfactant at pH 5 to 8 is admixed with a solution of the anti-L-NGFR in a phosphate buffer. The phosphate salts are added in such amounts that the previously defined concentrations are obtained. Subsequently it is sterilized by filtration and the solution prepared in this manner is lyophilized. After lyophilisation one obtains an optically perfect lyophilisation cake. The antibody anti-L-NGFR remains stable. After reconstitution of the lyophilisate with water for injection purposes a clear solution is obtained.

Claims

1. Stable lyophilized pharmaceutical preparation of monoclonal or polyclonal antibodies containing a sugar or an amino sugar, an amino acid and a surfactant.

2. Preparation as claimed in claim 1, wherein the preparation is essentially free of polyethylene glycols and/or free of protein-like standard pharmaceutical auxiliary substances.

3. Preparation as claimed in claim 1 or 2 composed essentially of
   a) a monoclonal or polyclonal antibody
   b) a sugar or amino sugar
   c) an amino acid
   d) an inorganic acid acting as a buffer substance and
   e) a surfactant.

4. Preparation as claimed in one of the claims 1 -3, wherein the sugar is a monosaccharide, disaccharide or trisaccharide, preferably sucrose, maltose, trehalose or raffinose.

5. Preparation as claimed in one of the claims 1-4, wherein the amino sugar is glucosamine, N-methyl-glucosamine, galactosamine or neuraminic acid.

6. Preparation as claimed in one of the claims 1 to 5, wherein the amino acid is a basic, acidic or neutral amino acid, preferably arginine, lysine, histidine, ornithine, isoleucine, leucine, alanine, glutamic acid or aspartic acid.

7. Preparation as claimed in one of the claims 1 to 6, wherein the surfactant is a polysorbate or a polyoxyethylene-polyoxypropylene polymer.

8. Preparation as claimed in one of the claims 1 to 7, wherein it contains physiologically tolerated auxiliary substances from the group comprising acids, bases, buffers and/or isotonizing agents.

9. Aqueous pharmaceutical preparation of monoclonal or polyclonal antibodies obtainable by redissolving the lyophilisate as claimed in one of the claims 1 to 8.

10. Aqueous pharmaceutical preparation as claimed in claim 9, wherein the solution has a pH value of 5-8, preferably of 6 - 7.4.

11. Process for the production of a lyophilized pharmaceutical preparation as claimed in one of the claims 1 to 8, wherein an aqueous preparation is produced that contains a monoclonal or polyclonal antibody as the active substance and a sugar or amino sugar, an amino acid and a surfactant as additives as well as optionally further pharmaceutical auxiliary substances and subsequently the solution is lyophilized.

12. Use of a combination of auxiliary substances composed of a) a sugar or an amino sugar, b) an amino acid and c) a surfactant for the production of stable therapeutic or diagnostic agents containing antibodies.

Abstract

The invention concerns lyophilized pharmaceutical preparations of monoclonal or polyclonal antibodies which contain a sugar or an amino sugar, an amino acid and a surfactant as stabilizers. In addition the invention concerns a process for the production of this stable lyophilisate as well as the use of a sugar or amino sugar, an amino acid and a surfactant as stabilizers for therapeutic or diagnostic agents containing antibodies.

N, M, M & O Docket No. P8341-9011 . NIKAIDO, MARMELSTEIN, MURRAY & ORAM LLP

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention **entitled**
(Insert Title) STABLE LYOPHILIZED PHARMACEUTICAL SUBSTANCES FROM MONOCLONAL OR POLYCLONAL ANTIBODIES
the specification of which is attached hereto unless the following box is checked:

☒ was filed on 19 November 1997 as PCT International Application Number PCT/EP97/06452 and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

				Priority Claimed
(List prior foreign applications. See note A on back of this page)	96118489.2 (Number)	EP (Country)	19 November 1996 (Day/Month/Year Filed)	☒ Yes ☐ No
	(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
	(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

(See Note B on back of this page) ☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

13

And I hereby appoint as principal attorneys: David T. Nikaido, Reg. No. 22,663; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Robert B. Murray, Reg. No. 22,980; E. Marcie Emas, Reg. No. 32,131; Douglas H. Goldhush, Reg. No. ~~33,125;~~ Monica Chin Kitts, Reg. No. 36,105; Richard J. Berman, Reg. No. ~~39,107;~~ King L. Wong, Reg. No. 37,500; ~~Karen~~ K. Costantino, Reg. No. 35,107; James A. Poulos, III, Reg. No. 31,714; Herbert C. Rose, Reg. No. 29,846; and Patrick D. Muir, Reg. No. 37,403.

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~~(202) 638-5000~~ Fax: (202) 638-4810

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

Full name of sole or first inventor: ~~Georg~~ KALLMEYER ~~KALLMEYER~~
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Rev. #15 2/22/99

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Inventor's signature _____ Date

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Citizenship: German

Post Office Address: Same as above

3-D

Full name of third joint inventor, if any: Christian KLESSEN

Inventor's signature Christian Klessen May 31, 1999 Date

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Citizenship: German

Post Office Address: Same as above

Full name of fourth joint inventor, if any: Heinrich WOOG

Inventor's signature _____ Date

Residence: Lindenstrasse 6, D-69514 Laudenbach, Germany

Citizenship: German

Post Office Address: Same as above

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention **entitled**
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				☐ Yes ☐ No
				☐ Yes ☐ No

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(Application Number)	(Filing Date)

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(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

And I hereby appoint as principal attorneys: David T. Nikaido, Reg. No. 22,663; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Robert B. Murray, Reg. No. 22,980; E. Marcie Emas, Reg. No. 32,131; Douglas H. Goldhush, Reg. No. 33,125; Monica Chin Kitts, Reg. No. 36,105; Richard J. Berman, Reg. No. 39,107; King L. Wong, Reg. No. 37,500; Karen K. Costantino, Reg. No. 35,107; James A. Poulos, III, Reg. No. 31,714; Herbert C. Rose, Reg. No. 29,846; and Patrick D. Muir, Reg. No. 37,403.

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(See Note C on back of this page)

Full name of sole or first inventor: Georg KALLMEYER

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2-00

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Inventor's signature _____ Date

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Post Office Address: Same as above

Full name of third joint inventor, if any: Christian KLESSEN

Inventor's signature _____ Date

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Citizenship: German

Post Office Address: Same as above

4-00

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